Report

on

THIRD TECHNICAL ALFALFA CONFERENCE

Western Regional Research Laboratory

Albany, California

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Western Utilization Research Branch

Agricultural Research Service

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and

American Dehydrators Association

PROGRAM

10:00 a.m.	Introduction	Dr. M. J. Copley, Chief Western Utilization Research Branch.
10:10 a.m.	Stabilization of Carotene in Alfalfa by Antioxidants	Dr. C. R. Thompson, Western Utilization Research Branch
10:30 a.m.	Vitamin E and Encephalomalacia in Chicks	Dr. George Kohler, National Alfalfa Dehydrating and Milling Co., Kansas City, Kansas
10:50 a.m.	Studies on Vitamins E, K and Carotene in Alfalfa	Dr. L. G. Blaylock, Colorado A and M College, Fort Collins Colo.
11:10 a.m.	Saponins in Alfalfa	Mr. E. M. Bickoff, Western Utilization Research Branch
11:30 a.m.	Estrogenic Factors in Forages	Dr. F. DeEds, Western Utilization Research Branch
2:00 p.m.	Present Status of Unidentified Growth Factors in Alfalfa	Dr. L. E. Card, University of Illinois, Urbana, Ill.
2:20 p.m.	Utilization of Alfalfa and Alfalfa Fiber by Animals	Dr. J. H. Meyer, University of California, Davis, Calif.
2:40 p.m.	Improved Alfalfa Seed Production	Dr. Ernest Stanford, University of California, Davis, Calif.
3:00 p.m.	Occurrence and Control of Yellow Clover Aphids	Dr. H. T. Reynolds, University of California, Riverside, Calif.
3:30 p.m.	Round Table Discussion	Moderator: Mr.Joseph Chrisman, American Dehydrators Association
4:00 p.m.	Adjournment	

Introductory Remarks, M. J. Copley

It is a pleasure for me to welcome you to the Third Technical Conference on Alfalfa. With its responsibility for doing research on improving the utilization of alfalfa, the Western Branch has profited very greatly from its contacts with the industry, and particularly with the American Dehydrators Association. We have learned much from such contacts about the problems and have been able, through the Association, to disseminate such useful information as we have been able to discover, as rapidly as possible to those who can best use it.

I thought that it might be worthwhile for me to spend a few minutes at this time in discussing our overall approach to alfalfa utilization research. Many people ask how we arrive at a research program on any specific commodity. The answer is that the problems are brought to us from several sources. In the case of alfalfa, meetings such as this one serve as one method of bringing research men and practical operators together. Another source of problems is the Department of Agriculture's Feed and Forage Advisory Committee, the members of which come from all parts of the country once a year to review our entire program on forages and to make definite recommendations for future work. We also welcome suggestions from individuals and, of course, from our own research people who, after being in the field for several years, are themselves competent to know what problems need to be attacked.

Our method of handling such problems may be illustrated with one which will be discussed by Dr. Thompson a little bit later this morning-- namely, that of preventing losses of carotene in dehydrated alfalfa during storage. Since carotene is one of the most valuable constituents of alfalfa it is obvious that it would be to the advantage of both grower and user to be able to sell- and to use- alfalfa in which the carotene content did not decrease during the winter months. This loss of carotene is due to oxidation of the pigment by air. It was known that the rate of loss could be decreased by storing the alfalfa in inert gas and also by refrigeration-- and, by the way, both of these methods are now in limited use--, but on the other hand, they were relatively expensive and, furthermore, they provided no protection for the alfalfa once it had left the inert-gas- or refrigerated warehouse. An entirely different possibility resided in the use of an antioxidant, that is, a chemical substance which could be added to the alfalfa to protect the carotene during subsequent storage. Studies directed toward this approach were begun more than 10 years ago and have continued up to the present. Several hundred substances were tested, and several with considerable protective capacity were discovered. These more promising ones were then examined from the toxicity standpoint in order to obtain evidence necessary for Food and Drug approval. Partly as a result of this activity several other laboratories initiated intensive work in the field and have come up with potentially valuable antioxidants. The result is that the carotene stability problem is very close to having a practical solution. The role of the government research program in this case is clear: by recognition of the problem and work in the

field, and by publication of its results, the government finds or stimulates others to find the solution. In the long run economic and technical factors will determine which solution, in this case which antioxidant, has the greatest merit. So much for the carotene in alfalfa. Dr. Thompson will fill you in with the present status of this problem.

You will also hear two reports this morning of our efforts in the field of composition of alfalfa. It has become obvious to us, after several years of wrestling with our commodities, that, we cannot do justice to their potential value without being thoroughly acquainted with their composition. We are therefore continually interested in accumulating information about the chemical substances present in alfalfa.

The work on saponins is cooperative with our colleagues in Beltsville who have been interested in the subject of bloat. In this case we prepare and study the saponins in legumes and they study their effects on ruminants. Any advance that can be made on the farm disease of bloat would markedly enhance the usefulness of legume forages.

Another field of immediate interest is that of the hormone-like flavonoid substances which occur in alfalfa. It is entirely possible that some of the unknown values of this crop, not yet directly attributable to known vitamins, etc. are due to the presence of these flavones. We are engaged in studies of these substances and Dr. DeEds will discuss the present status of our work.

In addition to these main lines of work, we support research that can be done more efficiently or expeditiously elsewhere. One of our contracts is with Colo. A & M College, and we are pleased that Dr. Blaylock could be with us to discuss the present status of their project on the vitamins of alfalfa.

This brief outline will, I hope, give you an outline of work and how we develop a program. We are pleased with this opportunity to tell you about it, and we want again to invite you to visit with us or write to us whenever you feel that we can be of assistance.

"Stabilization of Carotene in Alfalfa by Antioxidants" -- C. R. Thompson

Dehydrated alfalfa meal containing 100,000 I.U. of vitamin A (carotene) per pound has $17-20 per ton worth of the vitamin if it is considered on a par with vitamin A oils now being sold at 8.5 - 10¢ per million units. If alfalfa is stored for six months $10 - 12 per ton worth of vitamin is destroyed.

Much of this loss can be prevented by the addition of an antioxidant. Present results indicate that $7 - 9 worth of vitamin A value can be preserved by applying Santoquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) which cost $0.75 dissolved in oil or grease costing $0.75 - $1.50 per ton. This treatment

also renders the meal less dusty and preserves other nutrients such as vitamin E and possibly vitamin K and others.

The production of synthetic beta-carotene and stabilized vitamin A by Hoffman-La Roche Co. may have important effects on the price of vitamin A and perhaps dehydrated alfalfa. But to date synthetic A has depressed the price of vitamin A oils only slightly. This company has considered fortifying alfalfa meals with synthetic carotene or vitamin A if the price goes low enough.

In considering the advantages and disadvantages of using antioxidants for preserving carotene in alfalfa, four advantages may be listed:

1. Capital investment for antioxidant treatment is low i.e. much more is required for gas storage or perhaps refrigeration. It's especially simple if an oil is already being added to the meal.

2. The effect of antioxidant persists until the feed is consumed.

3. The antioxidant aids in preserving other fat soluble nutrients.

4. Some antioxidants may have a yet unexplained favorable effect on egg production in poultry.

The disadvantages of using antioxidants are as follows:

1. Antioxidants don't give quantitative preservation despite the use of high levels. The vitamin A loss may be reduced to one-third of its "normal" rate but not less.

2. Some antioxidants may be toxic.

3. The antioxidant may interfere in carotene analysis.

4. The antioxidant may have poor physical properties such as solubility.

Many antioxidants now approved for use in feeds or food are ineffective in this system. Tocopherols, N.D.G.A., BHA, propyl gallate and B.H.T. give little increase in carotene stability when added to alfalfa meal. This leaves two compounds, DPPD (N, N^1-diphenyl-p-phenylene-diamine) and Santoquin as alternatives. DPPD is poorly soluble in oils and is one-third to one-fifth as active per unit wt. as Santoquin. However, it is less expensive $1.18 /lb. as compared to $2.50 /lb. for Santoquin. It is also less toxic, being allowed at levels of 0.25 lb./ton in finished poultry feed while Santoquin is allowed at 0.3 lb./ton in dehydrated alfalfa.

The limitation of Santoquin to poultry feeds is preventing its use by many dehydration plants because brokers, blenders and feed manufacturers refuse to handle two kinds of alfalfa meal - one to be used only in poultry feed.

Members of the staff at the Western Regional Research Laboratory, Colorado A and M College, University of Illinois, and American Dehydrators Association have cooperated in studies designed to show if the compound was safe to use in all animal feeds. These have shown that Santoquin has very little toxicity to chicks, rats, calves and swine. Also, little accumulation of the chemical is observed in tissues. However, when dogs are fed the antioxidant toxicity is noted at about one-half the levels at which no effect is noted in rats. This has resulted in the Food and Drug Administration declaring it to be a poisonous and deleterious substance.

This agency also asked that experiments be conducted to show whether or not the material would appear in meat and milk when fed to dairy cows. Such experiments are planned and if the amounts appearing are sufficiently low, then a public hearing with the Food and Drug Administration will be petitioned for, and if granted tolerances for the use of the antioxidant will be established.

"Vitamin E and Encephalomalacia in Chicks" -- George O. Kohler

Nowadays, if a farmer should say to you, "Dig that crazy chick disease", you, of course, would know that he meant, "Look at that case of field encephalomalacia". There was a time you might not have known what he was talking about.

Feeding for broiler production is, today, a very different proposition than it was several years ago. The feed manufacturer used to be worried about protein, fat, N.F.E. and how much by-product he could load into a ration and still make the tag look presentable, and the feed look like feed rather than litter. Now, he must rely upon the expert advice of the nutritionist, in order to produce a saleable feed. Present-day feeds, with antibiotics, coccidiostats, new vitamins, "high energy" and even a pinch of arsenic, produce vastly superior growth and feed efficiency. But, the very changes which have helped to produce these amazing results may have helped to bring about a new crop of problems or to aggravate conditions previously of minor importance.

In discussing the relation of vitamin E to "crazy chick disease", I should first like to give you a general picture of encephalomalacia as it occurs in the field. Secondly, I should like to review the findings which have established that "nutritional encephalomalacia" is caused by a vitamin E deficiency. Thirdly, I will discuss the effects of antioxidants on nutritional encephalomalacia. Finally, I should like to present an alfalfa dehydrator's solution to the problem of nutritional encephalomalacia.

Field encephalomalacia is a disease of young chicks which is characterized by incoordinated movements and gait. In severe cases, the head is retracted, either forwards or backwards, and the feet are extended with toes flexed. These symptoms are caused by brain damage, localized, to a large extent, in the cerebellum or lower brain where muscular coordination is

centered. The brain damage seems to be related to a change in permeability of the capillaries, which leads to diffuse hemorrhage, sometimes seen in other parts of the body, as well as in the brain.

Incidence of the disease is maximum in flocks between three and five weeks of age. Practically none is seen in chicks over seven weeks old. A seasonal effect also exists, in that incidence is much greater during June and July than the rest of the year. Variation in yearly incidence will be discussed later.

While the field encephalomalacia was not a major cause of chick mortality, the nature of the outbreaks was such that the A.F.M.A. departed from its usual policy on grants-in-aid and supported a project at the University of Connecticut (Dr. Singsen, et al), aimed at determining causes and means of prevention.

So much for the general picture of encephalomalacia as it occurs in the field.

This brings me to the second phase of my discussion -- Vitamin E deficiency in chicks. It was shown many years ago by Dam and his co-workers in Denmark that one of the outstanding features of vitamin E deficiency in chicks is a change in cellular permeability with the accompanying tendency toward abnormal body fluid accumulations. Under some conditions, this led to nutritional encephalomalacia, described by Pappenheimer and Goetsch in 1931. Under other conditions, it led to a disease called exudative diathesis. In exudative diathesis, fluids accumulate in body tissues, between cells. For example, the skin around the crop may become so loaded with fluid that the chick appears to have a full crop, whereas the latter may actually be completely empty.

When Dam announced in 1938 that vitamin E would prevent nutritional emcephalomalacia as produced in the laboratory, many assumed that field "crazy chick disease" was the same condition since the symptoms and pathology were the same. Other nutritionists did not accept this assumption. One reason was that on the basis of the meagre data available, mixed commercial feeds contained much more vitamin E than had to be added to prevent the condition as produced in the laboratory. Another reason was that encephalomalacia could not be produced in the laboratory by feeding rations which had been fed to field chicks which had come down with the disease.

When the Connecticut workers undertook the work under the A.F.M.A. grant in 1951, they set up commercial-type rations from which the best vitamin E sources, including alfalfa meal, had been omitted. They found, as had the Danish workers, that no Vitamin E deficiency could be obtained. Only when cod liver oil was added to the rations did vitamin E deficiency appear. They also confirmed the other various conclusions of Dam, and added the important finding that maternal diet is a factor in producing encephalomalacia. Based on their work, the Connecticut workers have concluded

that field encephalomalacia is, indeed, the same as the laboratory-produced condition and is, therefore, a vitamin E deficiency.

They attribute the sporadic nature of outbreaks in the field to stress conditions which increase vitamin E destruction in rations, or increase vitamin E requirements of birds.

The ineffectiveness of the tocopherol in the practical ration ingredients was explained on the basis that the levels actually present were over-estimated by analytical procedures, and that some of the tocopherol actually present was not "available".

While the circumstantial evidence is strong that the field and laboratory conditions are identical, I would like to point out that direct proof of their identity is still lacking.

I would also like to take issue with recent conclusions of Singsen, et al, on the non-effectiveness of the vitamin E in alfalfa meal. Their conclusion was based on experiments in which alfalfa meal was added to their regular ration, containing 2 to 4% of cod liver oil. They found that alfalfa alpha tocopherol as measured by their chemical assay was not as effective under these conditions as was pure alpha tocopheryl acetate. This was interpreted as being due to poor availability. However, in another of their papers, the same workers report that free alpha tocopherol is not effective in this type of ration, while the acetate form is. Here, the difference was ascribed to the destructive effects of cod liver oil on the free form of tocopherol. I submit that the same effect caused the decreased response from alfalfa meal, which is known to contain the free, rather than esterified form, of the vitamin. Thus, the ineffectiveness of alfalfa meal under their laboratory conditions has no practical significance except to point up the fact that alfalfa meal, rather than cod liver oil, should be used as a source of vitamin A.

A still further criticism of the Connecticut availability work is that the conclusion is based on their analytical procedure giving correct results. There is reason to believe that the procedure they used was not accurate.

The American Dehydrators' Association is now sponsoring work at the Wisconsin Alumni Research Foundation to support these views. Preliminary results are not definitive as yet.

I should now like to proceed to the third part of my discussion, namely, the effects of antioxidants on encephalomalacia. One of the most interesting findings of Dam and his co-workers (1951) was that certain reducing compounds such as methylene blue, thionine and antabuse could prevent the occurrence of exudative diathesis and encephalomalacia as well as other vitamin E deficiency symptoms.

Kephart of National Alfalfa had found D.P.P.D. to be effective in stabilizing carotene and to be essentially non-toxic.

Putting these findings together, Singsen and his co-workers tested D.P.P.D. for its anti-encephalomalacia activity on the cod liver oil-type ration. They found it to be highly effective. Other vitamin E effects which have been found to be produced by D.P.P.D., methylene blue, antabuse or other reducing agents are listed as follows:

(1) Increased utilization of vitamin A in rats and chicks fed high cod liver oil rations. (Dam, et al, 1951; Singsen, et al, 1955.)

(2) Increased conversion of carotene to vitamin A (Kephart, 1947; Singsen, et al, 1954.)

(3) Increased utilization of xanthophylls by broilers to give improved shank pigmentation (Wilgus, 1955.)

(4) Increased transfer of xanthophyll to eggs in laying hens (Kephart, 1948.)

(5) Prevention of exudative diathesis (Dam, et al, 1951; M.L. Scott, 1955.)

(6) Prevention of Sterility in rats fed E deficient diet (Dam, 1951.)

(7) Prevention of dietary liver necrosis in rats (Schwartz, about 1952.)

(8) Prevention of depigmentation of incisors of rats fed high levels of cod liver oil (Dam, et al, 1951.)

(9) Prevention of peroxidation and the yellow-brown discoloration of fatty tissues of chicks and rats fed high levels of cod liver oil (Dam, et al, 1951.)

The fact that all of these vitamin E deficiency symptoms could be prevented by non-specific reducing agents which are not related chemically in any way, brings up the question as to whether alpha tocopherol really has a specific vitamin function or acts as a non-specific reducing agent.

There are two pieces of data which make me believe that alpha tocopherol actually is a specific vitamin and that the reducing agents function by preventing losses of vitamin E in the feed, in the digestive tract and, perhaps, in the tissues as well. The first data is the observation of Dam, et al, (1952) that the muscle degeneration seen in chicks fed low vitamin E, low fat diets can be prevented by alpha tocopheryl acetate but not by methylene blue or antabuse.

The other experiment which indicates that vitamin E is actually a vitamin and not a non-specific reducing agent was carried out by Dr. M. L. Scott of Cornell University. He found that D.P.P.D. was ineffective in preventing exudative diathesis in chicks fed a ration devoid of vitamin E. When low levels of free alpha tocopherol were added to the ration, a further addition of D. .P.D. prevented symptoms. Thus, D.P.P.D. acts either by stabilizing free alpha tocopherol already present in the ration, or by reducing the vitamin E requirements of the bird. It cannot completely replace vitamin E.

So much for the effects of antioxidants on encephalomalacia.

Before proceeding to the final phase of my discussion, I should like to summarize the situation up to this point.

Strong circumstantial evidence has been presented to show that field encephalomalacia is due to a vitamin E deficiency. This conclusion is accepted by most nutritionists. The practical solution to the problem, suggested by the University of Connecticut workers, is to add D.P.P.D. to the ration as a feed supplement.

An alternative solution suggested by Distillation Products is to add alpha tocopheryl acetate to commercial rations. It appears that this would cost at least as much as adding D.P.P.D. at present prices of tocopherol.

I should now like to give an alfalfa dehydrator's interpretation of the general situation, and finish up with a proposed plan of action. This is presented with full realization that some of the points are in need of verification by further research. I will therefore indicate, as I go along which items need more research.

(1) Field encephalomalacia is due to a vitamin E deficiency. (not quite proven.)

(2) The seasonal incidence peak of the disease corresponds, in time, to the minimum vitamin E content to be found in commercial samples of unguaranteed alfalfa meal.

(3) The decrease in incidence of field encephalomalacia from 1943-47 corresponds to the increased use of 100,000 unit guaranteed alfalfa meal.

(4) The increased incidence in 1948 corresponds to the increased use of "high energy" type rations with decreased alfalfa meal content.

(5) The decreased incidence in 1949-50 and 51 as compared with 1948 corresponds to the increased use of alfalfa in broiler rations which came about as the result of outbreaks of "crazy chick disease" and field hemmorrhogic disease.

(6) The tendency for incidence to be higher now that the pre-1948 level is due (a) to greater growth rates attained in the birds causing a higher E requirement and (b) to not enough alfalfa being used.

(7) Unguaranteed alfalfa meal cannot be depended upon as a source of vitamin E, since E is low by auto-oxidation, as is carotene.

(8) Guaranteed 100,000 unit alfalfa meal is a much more dependable source of vitamin E, since vitamin E is more stable in alfalfa than carotene. (this needs more work).

(9) The vitamin E of alfalfa meal is readily available, but is destroyed by added cod liver oil. (needs more confirmation.)

(10) The xanthophyll of alfalfa gives alfalfa added value as an anti-encephalomalacia supplement, since xanthophyll has been shown by Goldhaber et al to have a vitamin E sparing action. (needs confirmation.)

(11) Over 60% of the vitamin E in the average broiler ration is supplied by 5% of 100,000 unit guaranteed alfalfa meal. The remaining 40% can be relied upon only if fresh, high quality grain is used.

(12) Adding D.P.P.D. to the ration in dry form as a feed supplement is wasteful. Superior results can be obtained by adding much less D.P.P.D. in solution at the critical point, the alfalfa meal. This has the additional advantage of preserving the carotene, vitamin E and xanthophyll during storage of both the alfalfa and the feed. (Some data is available on carotene absorption, xanthophyll absorption and efficiency of carotene conversion to vitamin A. Other phases need more work.)

Based on these facts (and, perhaps, in some cases fancies), the alfalfa dehydrator might propose the following program to the feed manufacturer to give him the added protection from vitamin E deficiency which he desires--

(1) Use at least 3 to 5% of guaranteed 100,000 unit or better alfalfa meal as a cource of vitamin A, E, K and xanthophyll. Avoid cod liver oil. Other sources of vitamin A are unnecessary.

(2) Use only alfalfa meal stabilized with .04% D.P.P.D. or 0.015% Santoquin.

(3) Be prepared to pay a premium of $5 to $10 per ton for stabilized, guaranteed alfalfa meal. This will give the alfalfa dehydrator the margin he needs to do a good job and put out a premium product. The cost will still be less than adding either D.P.P.D. or alpha tocopherol acetate to the ration as individual supplements.

In closing, I wish to state in clear-cut terms that the above ideas are strictly personal and do not represent the official views of anyone but myself. I am presenting them in the hope that they may stimulate others to criticize, to get the needed research under way, and to help develop a constructive program for the dehydration industry.

"Studies on Vitamins E, K and Carotene in Alfalfa" -- Lynn G. Blaylock

On January 20, 1955, a contract was negotiated between Colorado A & M College and the Western Utilization Research Branch. The purpose of this contract was to initiate studies on the amounts of vitamins E, K and carotene in alfalfa, and to determine what happens to these vitamins when alfalfa is stored. We know what happens to carotene and therefore, it will be used as a guide to storage problems. Dr. W. E. Pyke, of the Chemistry Department has been designated as coordinator of this project. The assays for vitamin E and carotene are being carried out in Dr. Charkey's Laboratory in the Chemistry Department. The vitamin K assays are under my direction in the Poultry Department. This division of work was made because of the necessity for conducting a biological assay for vitamin K. Chicks are being used as the assay animal.

The project is set up so that 12 lots of dehydrated alfalfa, the 1st and 3rd cutting from each of six dehydrating areas, will be assayed on arrival for vitamin K by chick assay and for vitamins E and carotene by chemical methods. A portion of each of these samples is being stored for 12 and 24 weeks without any further treatment. The approximate storage temperature is 75°F. The relative humidity ranges from 10 - 40%.

Previous work has shown that treatment of alfalfa with antioxidants in oil would protect carotene during storage. Because of this it was decided that tests should also be made on the effects of antioxidants on the E and K content of alfalfa during storage. For purposes of carrying this out 3 samples of the 1st cutting and 3 from the 3rd will be treated with Santoquin (6--Ethoxy--1,2--dihydro--2,2,4--trimethyl quinoline) and stored for 12 and 24 weeks. These samples can then be compared with the untreated samples which have been under the same storage conditions. Similarly several samples of alfalfa will be treated with DPPD (N,N'--diphenyl--p--phenylene diamine) and handled in the same manner. Each of these antioxidants will be added to the alfalfa using 1% cottonseed oil containing the equivalent of 0.015% of the antioxidant.

One additional storage treatment will be used. Samples will be stored under nitrogen for 12 and 24 weeks, assayed at the end of each of these times and again they may be compared with the other storage treatments.

It is always interesting to know how one product compares with another and therefore 6 samples of sun-cured meal obtained from these same areas will be assayed. These samples will not be subjected to any further storage treatments.

In order to check on how the test samples compare with commercial samples, 10 lots of dehydrated alfalfa will be secured from regular trade channels. These samples will be assayed for each of the three vitamins as they come in. This will give you some idea of the design of the experiment.

Mr. Crisman of the American Dehydrators Association has arranged for us to secure samples of the first and third cuttings of alfalfa from the following areas: Dixon, California; Sherman, Texas; Lexington, Nebraska; Gray Town, Ohio; Johnstown, Colorado; and Heber, California. We have received the 1st cutting from all areas except Heber. This will give us samples from the main alfalfa producing areas and should tend to show whether there are any wide variations in the amounts of carotene, E and K in alfalfa.

I am sorry that I will not be able to report any data to you today on the amount of vitamins E and K in the various samples of alfalfa. The only data which I have are some preliminary carotene analyses which were made on the 1st series of samples to come in. These ranged from 115 to 232 micrograms of carotene per gram of alfalfa. It would appear from these values that we are getting good samples of alfalfa, and it will be interesting to see how much E and K the alfalfa contains and whether the amounts of these are changed by storage or by antioxidant addition.

"Saponins in Alfalfa" -- E. M. Bickoff

Saponins are naturally occurring glycosides which have soap-like properties and also display considerable physiological activity. In general, they have the ability to dissolve red blood corpuscles, poison fish and the lower animals, and to irritate the eyes, nose and mouth. In addition, some of the saponins isolated from alfalfa have the ability to inhibit chick growth, cause bloat in ruminants and inhibit peristalsis in isolated rabbit intestine.

Saponins may be split by acids or enzymes into sugars of various kinds plus a residue called a sapogenin. The sapogenin or aglycone may be either steroidol or triterpenoid. Those isolated from alfalfa saponin have the triterpenoid structure.

In order to determine whether saponin acts as a growth inhibitor for chicks, feeding experiments with purified alfalfa saponin were undertaken by Burt W. Heywang of the Bureau of Animal Industry, U. S. Dept. of Agriculture. The results showed conclusively that alfalfa saponin added to the diet at a level of 0.2% inhibits the growth of chicks.

Saponin has long been suggested by various investigators as being a contributing factor in causing ruminant bloat, especially "frothy" bloat. However, its exact role has been open to speculation for a number of years.

Animal testing with alfalfa saponin was undertaken at Beltsville by members of the Animal & Poultry Husbandry Research Branch under the direction of Dr. N. R. Ellis. In the first 10 tests in which alfalfa saponin was administered to ruminants, definite distension of the rumen was obtained in 8 cases. In all cases, the distension appeared to be due to gas retention rather than froth, since the passage of a stomach tube into the rumen permitted an immediate release of gas and reduction of distension. In more recent experiments, bloat has been produced in sheep by injection of alfalfa saponin directly into the blood stream. Studies of the muscular action of the rumens of the test animals show that the saponin injection causes a decrease in rumen motility and interferes with belching.

The Pharmacology Section of the WURB has studied the physiological response of isolated rabbit intestinal strips and have found that saponin causes a pronounced interference with the normal peristaltic behaviour.

A method has been developed to measure the total amount of saponin present in alfalfa. The amount present is quite variable in different samples of meal, ranging from 0.2 to 1.8% of the dry weight of the alfalfa.

There are at least seven different saponins in alfalfa; three of which are similar to saponins previously found only in soybeans. Preliminary results indicate that they may vary in their physiological effects upon animals.

We are now engaged in attempting to separate these saponins in pure form to permit their characterization and evaluation.

Studies are being planned to determine whether factors such as variety, cultural practice, or stage of growth have any bearing on the amount of saponin present.

There are four possible ways in which we might be able to overcome the harmful effect of saponins: (1) harvest at a time when saponin is low (2) treat the plants with some type of chemical to keep saponin low (3) treat animals in some manner so they are not affected by saponin or, (4) new varieties of alfalfa with low saponin contents may be developed.

"Estrogenic Factors in Forages" -- Floyd DeEds

There now exists a considerable body of evidence that certain forage crops, particularly the leguminous crops, are associated with such seemingly diverse phenomena as increased growth, increased food efficiency, improved lactation, loss of fertility, and bloat. The presence of estrogens in forages could account for all these phenomena with the exception of bloat.

An estrogen is a sex hormone and one is apt to think that its production and its physiological effects are limited to animal life. However, both estrone and oestriol, sex hormones found in the urine of pregnancy, have been isolated from plant materials. Beginning in 1953 and continuing to the present a number of investigators have shown the presence of estrogenic activity in forage materials. The genistin, genistein, and daidzein of soybean oil meal, and the biochanin in red clover have been shown to be estrogenic.

A systematic attack on the problem of isolating and identifying the chemical compounds responsible for estrogenic activity in ladino clover and several samples of alfalfa is in progress at the Western Utilization Research Branch. The facilities of the Pharmacology Section are being used to run the bioassay determinations of the estrogenic potency of the fractions prepared by the Field Crops Utilization Section. The results of the bioassays help to keep the chemists "on the beam" and guide them to the production of fractions of higher and higher potency.

At this point a few words concerning the bioassay procedure are in order. When a substance having estrogenic activity is administered to immature female mice or rats, the size and weight of the uterus increases rapidly as in pregnancy. The same result occurs in adult females in which the ovaries have been removed when an estrogen is given. Groups of weanling female rats are placed on an adequate basal diet, and on the same diet plus various percentages of the forage crop fraction to be tested. The rats are maintained on these diets for four or five days and then sacrificed, and the uteri of all animals removed and weighed. The increase in size and weight of the uteri of any experimental rats as compared with the uteri of the control rats on the basal diet alone is a measure of the estrogenic potency of the material tested. By applying the same procedure to crystalline compounds that may be eventually isolated, and comparing the increase in uterine weight produced by a given weight of material with that produced by a known amount of stilbestrol, some idea of absolute potency is obtained.

Some idea of the progress made in fractionating ladino clover pellets and concentrating the estrogenic activity may be obtained from the following data. The original pelleted material requires the ingestion of 8,000 milligrams to produce a 5 milligram increase in weight of the immature female rat uterus. We now have a fraction which causes a 41 milligram increase in uterine weight when 36 milligrams of material are ingested. We are looking forward to the isolation and identification of the active constituent, or constituents.

"Present Status of Unidentified Growth Factors in Alfalfa" -- L. E. Card

Experimental findings continue to support the belief that alfalfa contains unidentified growth factors but clues to their identity still escape detection.

As they have in the past, the opposite effects of growth-promoting and growth-depressing substances, both of which appear to be present in alfalfa, continue to make planning of experiments and interpreting of results difficult and uncertain.

Experimental work at the University of Illinois has continued to follow the general plan established some time ago. Cross-bred male chicks raised to an age of 28 days under controlled conditions and fed rations comprised of a semi-purified basal diet plus experimental supplements provide the data from which further information concerning unidentified growth factors in alfalfa is derived.

In general, additions to the basal diet of moderate proportions of alfalfa bring about increases in chick growth, but high levels of alfalfa lead to growth depression which becomes progressively greater as the proportion of alfalfa is increased beyond the optimum level. Experiments have shown that part, but not all, of growth depression that accompanies feeding of alfalfa at high levels is attributable to lessened feed intake.

In attempts to discover whether growth factors are concentrated in legume seeds, it was found that additions to the basal diet of either alfalfa seed or white clover seed at levels up to 0.8% neither stimulated nor depressed chick growth. Related experiments demonstrated that when 5% of white clover blossoms were added to the basal diet definite growth stimulation occurred and that growth depression was observed at the 20% level but not at the 10% level.

Relationship between plant maturity and chick growth response was examined by feeding the basal diet alone and supplemented with 5%, 10%, 20% and 40% of each of three lots of alfalfa cut respectively in the prebud-, but- and full blossom stages. Prebud alfalfa stimulated growth at the 10% level and did not depress growth at any of the levels at which it was fed. When fed at 5% level alfalfa cut at bud stage produced growth stimulation, but caused growth depression to appear at the 20% level. At 40% of the diet this alfalfa caused severe growth depression. Diet supplements comprised of alfalfa cut at full blossom produced in general the same chick growth responses as did those of alfalfa cut at bud stage except that growth depression began at the 10% supplement level.

Inconclusive results were obtained when normal alfalfa meals and meals that had been repeatedly extracted with hot water were used as diet supplements in attempts to learn whether the unidentified growth factors could be removed from the meals by leaching. Both types of supplements stimulated growth when fed at 5% and 10% levels but depressed it at high levels.

In experiments conducted this year cholesterol and soy oil were added alone and in combination to chick rations comprised of the basal diet supplemented at several levels with alfalfa. This was done in order to see whether the results would support the suggestions that saponin in alfalfa acts as a growth inhibitor and that its action may be overcome or lessened by co-administration of cholesterol. Data from these experiments follow:

Experiment 346 (1955)
28-Day Weights

Alfalfa	Basal	1% Cholesterol	4% Soy Oil	Both
None	453	441	477	387
5%	478	485	503	495
10%	470	481	467	507
20%	425	473	425	497
40%	287	384	307	420

Drackett protein - cerelose diet
Bud stage alfalfa - Sample L
3 replicates - 10 Males/lot.

The data illustrate the characteristic growth stimulation that occurs when the basal diet is supplemented with moderate proportions of alfalfa and the growth depression which results when high levels of alfalfa are included in the rations. It is also evident that addition of neither 1% of cholesterol nor 4% of soy oil completely overcame the adverse effects of high proportions of alfalfa. No explanation has been found for the growth depression which occurred when both cholesterol and soy oil were added to the control diet.

Existing confusion in the status of our knowledge of the unidentified growth factors in alfalfa rests largely upon our present inability to estimate them analytically or to isolate them from the rest of the plant material.

"Utilization of Alfalfa and Alfalfa Fiber by Animals" -- James H. Meyer

There are many reasons for studying the fiber fractions of alfalfa in terms of their utilization by simple-stomached and ruminant animals. In general it can be stated that the cheapest dry matter yield per acre can be obtained from roughage and in this case, alfalfa probably surpasses most other crops in terms of nutrient and protein yield. The California Experiment Station has data showing that it is possible to obtain gains as high as two pounds a day on alfalfa fed alone to steers for as long as a 170-day feeding period. In addition the University of Nevada has shown that high quality alfalfa is of great value in swine rations when it is fed as high as 50% of the ration. These two sets of data from ruminants and simple-stomached animals show that alfalfa can be an excellent source of energy, but it is also well known that most farmers do not get this type of utilization. This all leads to the much neglected portion of alfalfa - the fiber.

Knowledge of the crude fiber content of alfalfa is not enough because of the uncertain nature and value of this component. This component has an uncertain nature because it does not contain or show all the fiber that is present in roughage. For example, only part of the hemicellulose (most easily

digested portion of the fiber) is found in the crude fiber and in addition, lignin (the most indigestible portion of fiber) is only partially found in the crude fiber. Therefore, a proper study of roughages includes one in which the chemist actually determines cellulose, hemicellulose, and lignin. Hay quality standards need to bear a more direct relationship to the nutritive value of the feed but efforts in this direction have not been very successful because of lack of knowledge in this important field.

Investigations with rats at the University of California were made on fiber fractions isolated from alfalfa; namely, holocellulose (cellulose and hemicellulose), lignin, and an extracted alfalfa fraction which was that part remaining after alfalfa was extracted with hot water and a hot alcohol benzene solution. These residues were fed to rats to determine the extent of the utilization through the criteria of weight gain and nitrogen retention. The data showed that lignin was not utilized. Holocellulose was shown to be a poor energy source and did not support weight gains. The soluble fraction of the alfalfa was found to be primarily responsible as an energy source for animals and in addition was quite a good protein source. The fraction remaining after the extraction was not utilized to any great extent for energy, but it was found that the protein remaining in this fibrous fraction was available and utilized by the animal. This indicates that if alfalfa is to be utilized as an energy source, it is important that the alfalfa be very low in fiber and high in soluble constituents. There are some indications that this low fiber may not be necessary for proper utilization of the protein. However, more work has to be done before this question could be settled.

Alfalfa's main importance for ruminants is as a source of energy and here again the fiber components influence this to the greatest degree. Work conducted with artificial rumens in which the microorganisms were removed from live sheep and incubated in the laboratory, shows that a product such as safflower hulls was not digested to any great extent by the microorganisms. However, when the lignin was removed by treatment with sodium chloride, 150% increase in digestion of the hulls occurred. It was also shown in this work that grinding the hulls to a very fine powder did not influence digestibility to any great extent. This work confirms investigations by other stations that lignin, itself, is not only indigestible but renders other nutrients unavailable to microorganisms of the rumen.

Other experiments at the University of California have shown that the digestibility of alfalfas can vary to a small extent and still show very great differences in their influence on weight gains of steers. For example, it was found that the TDN of alfalfa fed green was 55.4 whereas this same green alfalfa harvested as hay and fed as hay had a TDN of 54.9. However, the animals gained 16.6 pounds for every 100 pounds of TDN consumed from green alfalfa but only gained 12.6 pounds for every 100 pounds of TDN from the hay. This indicates that for proper evaluation of alfalfa whether fed as pasture, soilage (green feed), or hay, digestibility does not tell the whole story. In this same study it was found that animals grazing on pasture gained 23 pounds per 100 pounds of TDN intake. This indicates that animals can select a forage

which has a nutritive content practically equal to that of concentrates. Therefore, it behooves scientists and ranchers to try to measure up to the standards set by the animal and produce alfalfa pasture or hay that is as good as the steer, himself, can select if given the opportunity. Being able to do this would increase very greatly the production of total nutrients per acre.

In summary, it can be stated that investigations conducted to date by experiment stations have only scratched the surface in their study of alfalfa or other roughages. However, it has been shown that lignin, cellulose, and hemicellulose are not utilized by the simple-stomached animals even though they may disappear to a certain extent in the digestive tract. Cellulose and hemicellulose are utilized by the ruminants but their utilization is influenced to a very great extent by the lignin content of the roughage. Lignin is not utilized for energy purposes and in addition prevents the utilization of the other nutrients such as protein and cellulose by sheep or cattle.

"Progress in Alfalfa Improvement" -- E. H. Stanford

Breeding work on alfalfa--for that matter on forage crops in general--has only been carried on to any extent for about the last 25 years in the United States. In contrast, intensive work on cereal breeding has been carried on for more than 50 years. As a consequence, new bred varieties of alfalfa have come into prominence only during the last ten years. I will outline briefly the work under way in California and mention the varieties which have been produced in other states.

In California our attention has been directed primarily toward breeding for disease and insect resistance. We have felt that this approach would give the most rapid improvement in productivity and quality. As an example, breeding for leafspot resistance has given better quality hay on spring cuttings when leafspot was severe. Analyses of samples taken from plots under heavy infection are given below:

Severe Leafspot Infection

	% Leaves	% Protein	Carotene p.p.m.
California Common	38	12.9	96
Caliverde	51	18.4	204

Bacterial wilt is the most important disease in the state and the one to which we have given the most attention. We had to go to an introduction from Turkistan as a source of our resistance. We transferred resistance to our California Common type by crossing and backcrossing to Common. We also combined leafspot and mildew resistance in the program. The result is the variety Caliverde, combining resistance to these three diseases.

The dwarf virus disease is perhaps next in importance in the San Joaquin Valley and in Riverside County. We have selected (for resistance to this disease within the California Common Variety) to produce the variety California Common 49. Presently we are breeding to transfer dwarf resistance to Caliverde.

Other projects under way include breeding for Rhizoctonia root canker and crown rot, particularly for the Imperial Valley. We believe we have made definite progress in selecting for resistance or tolerance to this disease.

The variety Lahontan, developed in Nevada, has good resistance to the stem nematode; and we are recommending it for areas where the nematode is prevalent. We are in the process of transferring this resistance to the Caliverde variety.

Closely related to this program is the problem of the spotted alfalfa aphid. Lahontan has good resistance which we feel sure can be transferred to better adapted varieties.

We have also begun some selection work for resistance to Phytophthora root rot and for the root-knot nematode. We have found good resistance to the Nematode, but resistance to phytophthora is not so positive.

Breeding programs in other states have produced the varieties: Ranger, Buffalo, Atlantic, Narragansett, Williamsburg and Vernal, each with its own area of adaptation. Eastern states have not been able to produce seed supplies of these varieties. Research on factors affecting seed production has enabled us to expand our seed-producing industry very rapidly in the last ten years. Our production has increased from about 5 million pounds of seed annually to nearly 60 million pounds and is still increasing. We have found that by controlling harmful insects, providing plenty of pollinating insects and following proper management practices, we can be assured of a good seed crop every year in our good seed-producing areas. This has helped to stabilize the seed market and provide a constant supply of seed of the improved varieties for the Eastern growers.

"Occurrence and Control of Yellow Clover Aphids" -- H. T. Reynolds

The yellow clover aphid in California seems to be the same one that has lived on alfalfa in India and in the Mediterranean region for a long time. Whether this alfalfa-preferring yellow clover aphid is a distinct species, a subspecies, or a host preference strain is not known at the present time.

It is very probable that the yellow clover aphid in California was accidentally introduced into New Mexico about two years ago. From that center it has spread west through Arizona into California and north as far as Fresno. It has also spread east over Texas and north through Oklahoma into Kansas and Arkansas. It also prefers bur clover, sour clover and black medic but will not live on red clover, ladino clover, subterranean clover, vetch or birdsfoot trefoil.

After the yellow clover aphid was discovered in California on February 7, 1954, there was a surge of heavy population--a common occurrence with many new pests--but there seems to be a settling down to a less injurious annual pattern. By mid May 1955 the populations of the the yellow clover aphid in alfalfa fields in the desert areas of California had dropped off to such an extent that many fields did not require treatment. Localities nearer the coast have their population peaks shifted toward the summer. It appears that the yellow clover aphid may never be a pest in a true coastal climate.

The highest population counted in the current investigation amounted to about 600 aphids per stem of alfalfa, or more than one billion per acre. Short hay alfalfa begins to show stickiness at about 40 aphids per stem, or 70 million per acre. Populations injurious to seedling alfalfa may be much lower, possibly not more than one aphid per plant on very small seedlings.

An important fact in yellow clover aphid populations is the high percentage of winged forms produced. Stands have been ruined in a few days by aphids that have flown in from adjacent hay fields.

Yellow clover aphid feeding seems to be poisonous to the alfalfa plant. This is particularly evident in small seedling alfalfa. The little plants die suddenly from the feeding injury.

Damage to hay crops may take the form of retarded growth, stickiness from honey dew that interferes with harvesting and baling, or sometimes complete loss of quality from the dropping of the lower leaves and blackening by sooty mold fungus which grows on honeydew.

A special type of injury is prevention of regrowth after cutting.

Aphids may completely cover the developing shoots when they are about 1" long and stop their growth entirely. If allowed to persist, this situation may cause the death of many plants and seriously thin the stand as heavy aphid populations on large alfalfa may do. The aphid appears to be aided in stand thinning by soil fungi that invade weakened roots.

The yellow clover aphid in California goes by the scientific name, of *Therioaphis trifolii* (Monell), also called *Myzocallis trifolii* (Monell). It has become increasingly evident that the yellow clover aphid in California is not identical with the yellow clover aphid that has been present on clovers in eastern and midwestern United States for the past 80 years.

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NOT PRESENTED AS A TALK

"Influence of Several Factors on Conversion of Alfalfa Carotene to Vitamin A in the Chicken" -- H. J. Almquist and Stanley Maurer, The Grange Co., Modesto

Elevation of liver vitamin A and serum carotenoids in 26-day-old chicks fed a diet containing sodium penicillin G at a level of 30 mg./kg. has been reported by Burgess et al. (1) The diet used contained generous amounts of both carotenoid and fish-oil sources of vitamin A activity, hence it is not known whether the antibiotic affected the metabolism of one or both of the sources.

In experimental work conducted under practical rearing conditions with Indian River-New Hampshire Cross broiler chicks, we have measured the effects of dietary antibiotics, an antioxidant, and a fat on the deposition in the liver of vitamin A and carotene. The birds were fed a practical diet containing only plant sources of vitamin A activity, primarily dehydrated alfalfa meal, at a level of 5% of the diet. The approximate vitamin A activity of the diet was 11,000 I.U./kg. Aside from differences in supplements mentioned, the diets were identical. Vitamin A and carotene were determined on pooled livers from 6 birds selected as being representative of each group. The method of determination was essentially that given by Gallup and Hoefer (2).

Table I

Liver Vitamin A and Carotene of Broiler Chicks in Relation to Dietary Supplements of Antibiotic, Antioxidant, and Fat

Supplement to Diet, Per Lb.	Per Gram of Liver			
	Vitamin A Units		Carotene, Mcg.	
	8 Weeks	10 Weeks	8 Weeks	10 Weeks
None	45	83	2.6	5.6
Antibiotic,a/ 2 mg.	55	94	3.1	7.6
Chlortetracycline, 25 mg.	--	115	--	8.8
Chlortetracycline, 50 mg.	113	115	4.8	7.2
Antioxidant,b/ 114 mg. plus Antibiotic,a/ 2 mg.	71	89	3.3	6.8
Fat,c/ 1.43%, plus Antibiotic,c/ 2 mg.	85	103	3.1	6.6

a/ A mixture of chlortetracycline and diamine penicillin.
b/ N,N'-diphenyl-p'phenylenediamine.
c/ Rice bran oil

Liver vitamin A was increased slightly by the lowest level of antibiotic fed. The higher levels of antibiotic caused further elevation of liver vitamin A which may have reached a ceiling at the 55 mg. level. The same general results were obtained in respect to liver carotene content. Since conversion of carotene

to vitamin A occurs in the intestinal wall (3), it is possible that maintenance of a healthier intestinal condition in the presence of the antibiotic is responsible for improved conversion or absorption of carotene, or both.

The effect of the antioxidant, N,N'-diphenyl-p-phenylenediamine, appears essentially neutral when allowance is made for the low level of antibiotic also present in the diet. This fact is of interest in showing that there was no apparent interference with carotene conversion. The antioxidant, at this level, has been shown to exert a marked sparing or replacement action on vitamin E in the chick (4). High et al. (5) have found that a large intake of vitamin E or certain antioxidants will depress conversion of carotene to vitamin A in the rat.

Fat added to the diet at the moderate level of 1.43% consistently improved vitamin A deposition. The basal diet contained approximately 4% fat. The favorable effect of fat on carotene conversion has been reviewed and further demonstrated by Deuel et al. (6).

In subsequent studies on protein and energy levels in broiler diets we had an opportunity to obtain further information on the effect of added fat. As before, the diets were practical in nature and contained only dehydrated alfalfa meal for the primary source of vitamin A activity. Aside from differences in level of fat added the diets were identical. The fat was yellow grease from a rendering plant, of a grade suitable for use in poultry feeds. The diets contained the same amount of an antioxidant, DPPD, and the antibiotic mixture as previously used. Chicks were taken for analysis at 8 weeks of age. The same procedures were followed.

Table II.

Liver Vitamin A and Carotene of Broiler Chicks in Relation to Levels of Added Fat in the Diet

Fat Added to Diet		Per Gram of Liver: Yellow Pigments as B-Carotene	Per Gram of Liver: Vitamin A
%		mcg.	units
	Males		
0.1		8.8	131
2.1		15.6	147
4.2		10.0	145
5.8		10.0	129
	Females		
0.0		9.2	96
2.1		12.6	164
4.2		9.2	149
5.8		7.6	87

The above results are consistent with former work in showing that the added fat improved conversion of carotene to Vitamin A, as indicated by liver storage. There was also some slight increase in general yellow pigmentation of the carcass where fat was added to the diet. Size and color of livers were comparable among groups of the same sex.

The results indicate further that the highest level of added fat was interfering, relatively, with carotene conversion. It is well-known that high fat intakes in other animals may show the same apparent reversal of the effect of the lower fat levels.

(1) Burgess, H. C., Gluck, M., Brisson, G., and Laughland, D. H., Arch. Biochem. and Biophys., 33, 339 (1951).

(2) Gallup, W. D., and Hoeffer, J. A., Ind. Eng. Chem., Anal. Ed., 18, 288 (1946).

(3) Cheng, A. L. S., and Deuel, H. J., Jr., J. Nutrition, 41, 619 (1950).

(4) Bunnell, R. H., Matterson, L. D., Singsen, E. P., Potter, L. M., Kozeff, A., and Jungherr, E. L., Poultry Science, 33, 1046 (1954).

(5) High, E. G., Smith, H. C., Jr., Taylor, H. H., and Wilson, S. S., J. Biol. Chem. 210, 681 (1954).

(6) Deuel, H. J., Jr., Greenberg, S. M., Savage, E. E., and Melnick, D. J., Jour. Nutrition 43, 371 (1951).